

CLAIMS

1. *rpoB* gene or gene fragment of a bacterium of the genus *Streptococcus* and the 4 related genera *Enterococcus*,
5 *Gemella*, *Abiotrophia* and *Granulicatella*, characterized in that it comprises a sequence chosen from among sequences SEQ ID n° 8 to 35 in which:

- the K nucleotide represents T or G
- the M nucleotide represents A or C,
- 10 - the R nucleotide represents A or G,
- the W nucleotide represents A or T,
- the Y nucleotide represents C or T,
- the N nucleotide represents A, T, C, G or I, and

the reverse sequences and complementary sequences and those
15 sequences having at least 98.7 % homology, excepting sequences SEQ ID n°11, 12, 14 and 22.

2. *rpoB* gene of one of the bacteria *Streptococcus anginosus*, *Streptococcus equinus*, *Abiotrophia defectiva* and *Enterococcus faecalis* as in claim 1, characterized in that it
20 corresponds to one of the sequences chosen from among sequences SEQ ID n°1 to 3 and SEQ ID n° 5 in which:

- the K nucleotide represents T or G,
- the M nucleotide represents A or C,
- the R nucleotide represents A or G,
- 25 - the W nucleotide represents A or T,
- the Y nucleotide represents C or T,
- the N nucleotide represents A, T, C, G or I,

and the reverse sequences and complementary sequences and those sequences having at least 98.7% homology.

30 3. *rpoB* gene fragment of a bacterium of the genus *Streptococcus* and of the 4 related genera *Enterococcus*, *Gemella*, *Abiotrophia* and *Granulicatella*, characterized in that

its sequence is included in or consists of one of sequences SEQ ID n° 8 to 35, in which:

- the K nucleotide represents T or G,
- the M nucleotide represents A or C,
- 5 - the R nucleotide represents A or G,
- the W nucleotide represents A or T,
- the Y nucleotide represents C or T,
- the N nucleotide represents A, T, C or G

and the reverse sequences and complementary sequences,
10 and those sequences having at least 98.7% homology.

4. Oligonucleotide characterized in that it comprises a sequence specific to a species of a bacterium of genus *Streptococcus* and said related genera, preferably having at least 20 consecutive nucleotides, further preferably at least
15 30 consecutive nucleotides included in one of said sequences SEQ ID n° 8 to 35, in which:

- the K nucleotide represents T or G,
- the M nucleotide represents A or C,
- the R nucleotide represents A or G,
- 20 - the W nucleotide represents A or T,
- the Y nucleotide represents C or T,
- the N nucleotide represents A, T, C or G

and the reverse sequences and complementary sequences and those sequences having at least 98.7% homology.

25 5. Use of a gene, gene fragment or oligonucleotide as defined in any of claims 1 to 4 as species probe for a bacterium of genus *Streptococcus* and said related genera.

6. Oligonucleotide characterized in that it comprises a sequence of at least 8, preferably at least 12, further
30 preferably 18 to 35 nucleotide motifs, including at least one sequence of 8 consecutive nucleotide motifs included in one of the following sequences SEQ ID n° 6 and 7:

- SEQ ID n° 6: 5'- AARYTNGGMCCTGAAGAAAAT-3', and
- SEQ ID n° 7: 5'- TGNARTTTTRTCATCAACCATGTG-3'

in which:

- N represents inosine or one of the 4 nucleotides A, T,
5 C or G,
- R represents A or G,
- M represents A or C, and
- Y represents C or T,

and the reverse sequences and complementary sequences.

10 7. Mixture of oligonucleotides, characterized in that it consists of an equimolar mixture of oligonucleotides as defined in claim 6, all having a different sequence and all comprising a sequence included in SEQ ID n° 6 or all a sequence included in SEQ ID n° 7.

15 8. Mixture of oligonucleotides, characterized in that it consists of an equimolar mixture of 32 oligonucleotides as defined in claim 7, having different sequences and each comprising at least 15, preferably at least 18 consecutive nucleotide motifs, included in the following sequence:

20 - SEQ ID n°6: 5'- AARYTNGGMCCTGAAGAAAT-3'

in which:

- R represents A or G,
- Y represents C or T,
- M represents A or C, and
- 25 - N represents A, T, C or G

and the reverse sequences and complementary sequences.

9. Mixture of oligonucleotides, characterized in that it consists of an equimolar mixture of 8 oligonucleotides as defined in claim 7, having different sequences and each
30 comprising at least 15, preferably at least 18 consecutive nucleotide motifs included in the following sequence:

- SEQ ID n°6: 5'- AARYTNGGMCCTGAAGAAAT-3'

in which:

- R represents A or G,
- Y represents C or T,
- M represents A or C, and
- 5 - N represents inosine,

and the reverse sequences and complementary sequences.

10. Mixture of oligonucleotides characterized in that it consists of an equimolar mixture of 16 oligonucleotides as defined in claim 7, having different sequences and each
10 comprising at least 15, preferably at least 21 consecutive nucleotide motifs included in the following sequence:

- SEQ ID n°7: 5'- TGNARTTTTRTCATCAACCATGTG-3'

in which:

- R represents A or G, and
- 15 - N represents A, T, C or G

and the reverse sequences and complementary sequences.

11. Mixture of oligonucleotides, characterized in that it consists of an equimolar mixture of 4 oligonucleotides as defined in claim 7, having different sequences and each
20 comprising at least 15, preferably at least 21 consecutive nucleotide motifs included in the following sequence:

- SEQ ID n°7: 5'- TGNARTTTTRTCATCAACCATGTG-3'

in which:

- R represents A or G, and
- 25 - N represents inosine

and the reverse sequences and complementary sequences.

12. Mixture of oligonucleotides as defined in any of claims 7 to 11, characterized in that said sequences consist of sequences SEQ ID n°6 and 7 in which, preferably, N
30 represents inosine, and the reverse sequences and complementary sequences.

13. Use of an oligonucleotide or mixture of oligonucleotides as in any of claims 6 to 12, as amplification primer or genus probe for a bacterium of genus *Streptococcus* and said related genera.

5 14. Detection method by molecular identification to detect a bacterium of one of the species of genus *Streptococcus* and the 4 related genera *Enterococcus*, *Gemella*, *Abiotrophia* and *Granulicatella*, characterized in that the following is used:

- 10 - an *rpoB* gene or gene fragment or an oligonucleotide as in any of claims 1 to 4 and an *rpoB* gene or gene fragment of a bacterium *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Streptococcus mutans* and *Streptococcus agalactiae* comprising a sequence such as
- 15 respectively described in sequences SEQ ID n°11, 12, 14 and 22, and the reverse sequences and complementary sequences, and those sequences having at least 98.7% homology, and/or
- 20 - at least one oligonucleotide or mixture of oligonucleotides as in any of claims 6 to 12.

15. Method as in claim 14 in which it is sought to detect the presence of a bacterium of genus *Streptococcus* or of said 4 related genera, characterized in that it comprises the steps in which:

- 25 1-at least one genus probe comprising a said mixture of oligonucleotides as in any of claims 7 to 12, is contacted with a specimen containing or possibly containing nucleic acids of at least one such bacterium of genus *Streptococcus* and its said 4 related genera,
- 30 and
- 2-the formation or non-formation of a hybridisation complex is determined between said genus probe and the nucleic acids of the specimen, and in this way the

presence is determined of said bacterium in the specimen if a hybridisation complex is formed.

16. Method as in claim 14, characterized in that it comprises the steps in which:

- 5 1- the amplification primers comprising said mixtures of oligonucleotides as in any of claims 7 to 12 are contacted with a specimen containing or possibly containing nucleic acids of at least one such bacterium of genus *Streptococcus* and said 4 related genera, and
 - 10 with:
 - as 5' primer, a said mixture of oligonucleotides comprising a sequence included in sequence SEQ ID n°6, preferably consisting of said complete sequence SEQ ID n°6, or a said complementary
 - 15 sequence as in any of claims 7, 8, 9 or 12, and
 - as 3' primer a said mixture of oligonucleotides comprising a sequence included in sequence SEQ ID n°7, or preferably consisting of said complete sequence SEQ ID n°7, or a complementary sequence
 - 20 as in any of claims 7, 10, 11 or 12.
 - 2- the nucleic acids are amplified by enzymatic polymerisation reaction to determine the presence or absence of an amplification product, and in this manner the presence is determined of said bacterium in the
 - 25 specimen if an amplification product occurs.

17. Method as in claim 14 or 16, characterized in that it is sought to specifically detect a given species of a bacterium in the *Streptococcus* group and said 4 related genera, chosen from among the species:

30 *Streptococcus mutans*, *Streptococcus oralis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus salivarius*, *Streptococcus sanguinis*, *Streptococcus suis*, *Streptococcus acidominimus*, *Streptococcus agalactiae*, *Streptococcus*

anginosus, *Streptococcus constellatus*, *Streptococcus*
difficilis, *Streptococcus dysgalactiae*, *Streptococcus equi*,
Streptococcus equinus, *Streptococcus intermedius*,
Streptococcus mitis, *Streptococcus bovis*, *Streptococcus*
 5 *alactolyticus*, *Streptococcus gallolyticus*, *Streptococcus*
macedonicus, *Streptococcus infantarius*, *Streptococcus hominis*,
Granulicatella adjacens, *Abiotrophia defectiva*, *Enterococcus*
avium, *Enterococcus casseliflavus*, *Enterococcus faecalis*,
Enterococcus faecium, *Enterococcus gallinarum*, *Enterococcus*
 10 *sacharolyticus*, *Gemella haemolysans*, and *Gemella morbillorum*,
 method in which:

1- a specimen containing or possibly containing nucleic
 acids of at least one such bacterium is contacted with
 at least one species probe consisting of a gene or gene
 15 fragment as in any of claims 1 to 3, or an
 oligonucleotide as in claim 4, and
 2- the formation or non-formation is determined of a
 hybridisation complex between said probe and the
 nucleic acids of the specimen, and in this way the
 20 presence of said bacterium in the sample is determined
 if a hybridisation complex is formed.

18. Method as in claim 14, characterized in that it is
 sought to detect a given species of a bacterium of genus
Streptococcus and said related genera chosen from among the
 25 species:

Streptococcus mutans, *Streptococcus oralis*, *Streptococcus*
pneumoniae, *Streptococcus pyogenes*, *Streptococcus salivarius*,
Streptococcus sanguinis, *Streptococcus suis*, *Streptococcus*
acidominimus, *Streptococcus agalactiae*, *Streptococcus*
 30 *anginosus*, *Streptococcus constellatus*, *Streptococcus*
difficilis, *Streptococcus dysgalactiae*, *Streptococcus equi*,
Streptococcus equinus, *Streptococcus intermedius*,
Streptococcus mitis, *Streptococcus bovis*, *Streptococcus*

alactolyticus, *Streptococcus gallolyticus*, *Streptococcus macedonicus*, *Streptococcus infantarius*, *Streptococcus hominis*, *Granulicatella adjacens*, *Abiotrophia defectiva*, *Enterococcus avium*, *Enterococcus casseliflavus*, *Enterococcus faecalis*,
 5 *Enterococcus faecium*, *Enterococcus gallinarum*, *Enterococcus sacharolyticus*, *Gemella haemolysans*, and *Gemella morbillorum*,
 a method in which, in a specimen containing or possibly containing nucleic acids of at least one said bacterium of genus *Staphylococcus*, the steps are performed in which:

- 10 a) a sequencing reaction is conducted of an amplified *rpoB* gene fragment of a said given bacterium using nucleotide primers consisting of said oligonucleotide mixtures as in any of claims 7 to 12 comprising sequences included in sequence SEQ ID n°6 as 5' primer
 15 and in SEQ ID n°7 as 3' primer, preferably sequences consisting of said sequences SEQ ID n° 6 and 7, and said complementary sequences, and
- b) the presence or absence of the given species of said bacterium is determined by comparing the sequence
 20 obtained of said fragment with the sequence of the complete *rpoB* gene of said bacterium or the sequence of a *rpoB* gene fragment of said bacterium respectively comprising said sequences SEQ ID n°8 to 35 as in any of
 25 claims 1 to 4 and complementary sequences, and in this manner the presence of said bacterium in the specimen is determined if the obtained sequence of said fragment is identical to the known sequence of the *rpoB* gene or gene fragment of said bacterium.

19. Diagnosis kit for use in a method as in any of
 30 claims 14 to 18, characterized in that it comprises at least one said oligonucleotide, mixture of oligonucleotides, or gene fragment as in any of claims 1 to 4 and 6 to 12.